AMINO ACID COMPOSITION OF WHEAT AS RELATED TO DYE ABSORPTION

by

FLOYD KENT SHOUP

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Approved by:

C W Deyoe
Major Professor

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INTRODUCTION

It is necessary to continue to search for improved and more rapid procedures for estimation of amino acid content of the cereal proteins. The importance of knowing the amino acid content was pointed out in the fundamental studies of Osborne and Mendel (1914) which led to the realization that the nutritional value of protein is dependent upon the content of the amino acids required for growth, maintenance, and other metabolic functions. It is also important to have a knowledge of cereal proteins because their unique physical properties and contributions to kernel structure are significant factors in processing. Extensive technologies have been developed in milling, baking, malting and feed formulation which require a knowledge of the effect protein and its constituents have upon the product.

The purpose of this study was to investigate the relationship of dye binding capacity of intact wheat protein to the amino acid composition. Methods for rapid estimation of amino acid composition would result in faster and more economical procedures for estimating protein quality or processing characteristics.

Amino acid analyses were accomplished by acid hydrolysis and separation by ion exchange chromatography. Determinations were made for the following amino acids: lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, half cystine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine.

The capacity of the wheat protein to absorb dye was measured by using colorimetric methods to estimate absorption of acid orange 12. The procedure and equipment used was designed to estimate total protein content. Therefore, the procedure was considered to be appropriate for attempting to find relationships between dye absorption and the amino acid composition.

REVIEW OF LITERATURE

Comparisons of Amino Acid Concentration Between Wheat and its Flour

The amino acid composition of flour and wheat protein has for many years been compared to determine the effect of milling on the protein content. This was done in an attempt to define quality and to account for the differences found in various mill products. Barton-Wright, et al., (1946) studied the distribution of amino acids within the wheat grain. They found that apart from the germ there is a rising gradient of amino acids from the center to the outside of the grain. However, the concentration in the protein of the bran, with the exception of arginine, lysine, and tryptophan, is less than that in the center endosperm. Pence, et al., (1964) also reported differences in fractions of wheat. Greater proportions of lysine, glycine, arginine, alanine, and aspartic acid were found in fractions containing germ and bran. Concentrations of glutamic acid, proline, and phenylalanine were high in the flour fraction. Essentially the same thing was found by Hepburn, et al., (1960) when comparing the whole wheat and flour fractions. Hepburn, et al., (1957) reported the protein of flour contained less lysine (24%), arginine (19%), aspartic acid (19%), glycine (17%), alanine (14%), threonine (7%), and tryptophan

(6%) when compared to whole wheat protein. Substantial increases in glutamic acid (21%), proline (19%), phenylalanine (11%), isoleucine (9%), leucine (8%), serine (8%), cystine (7%), and tyrosine (5%) were also found. Horn, et al., (1958) also found these differences when they made an amino acid study of flour, bran, and shorts.

Effect of Variety on Amino Acid Concentration

Several studies have been conducted to determine differences in amino acid content of the protein as affected by varieties. Miller, et al., (1950), Pence, et al., (1950), Hepburn, et al., (1957), Lawrence, et al., (1958), and Simmons, (1962) have all studied the effect of variety and have concluded there are only small differences.

Effect of Protein Concentration on Amino Acid Distribution

The amount of the various amino acids in protein appears to change as the total protein content varies. That is to say, the percentage distribution of amino acids in cereal protein changes as the total protein content increases or decreases. This effect has been reported by several workers when comparing lysine level to protein content. Lawrence, et al., (1958) found the lysine content of protein to be a function of the logarithm of the total protein. An equation was calculated for the regression line when plotting percent lysine in protein versus percent protein. A regression line calculated for 143 samples containing less than 13.5% protein had a correlation coefficient of 0.73. The correlation coefficient was nonsignificant, however, between lysine and protein for samples containing more than 13.5% protein. McDermott and Pace (1960), McElroy et al., (1949), Simmons, (1962) and Price (1950) also reported an inverse relationship between the lysine content and percentages of protein. Hepburn and Bradley (1965) observed the amounts of several amino acids showed an inverse relationship to the protein content. However, it was noted that glutamic acid, phenylalanine, and proline varied as the protein varied. Histidine, isoleucine, and tyrosine had the least tendency to be related to protein content. Miller, et al., (1950) reported differences in the percentages of cystine and methionine in wheat protein due to environment. Similar differences were not observed with lysine or glutamic acid. It was also noted that wheat having greater amounts of cystine in the protein required a longer mixing time for optimal dough development.

Acid Hydrolysis

Before an amino acid analysis can be carried out, hydrolysis or cleavage of the peptide bonds of the protein is necessary. The procedure for sample preparation does not remove the carbohydrate portion of the kernel before hydrolysis takes place. The effect of large amounts of carbohydrate on the recovery of amino acids from protein of cereal grains has not been extensively examined. Roxas (1916) reported some evidence which showed that the α-amino groups of arginine, histidine, lysine, and tryptophan take part in the reaction with sugars. It was also shown that in tyrosine the reactive group is presumably the hydroxyl group and surely not the α-amino group. In cystine the α-amino group remained intact so that presumably the reaction was with the mercaptan group.

Arginine, histidine, and tryptophan was found to react more readily with sugars in weak acid than in strong acid solutions. Dustin (1953) reported the recovery of amino acids after acid hydrolysis was in no instance lowered more than 3% by addition of carbohydrates. One to two gram protein samples were hydrolyzed in the presence and absence of carbohydrate for 22 hours with 200 ml of 6 N hydrochloric acid. The amino acids recovered were aspartic acid, threonine, serine, glutamic acid, alanine, glycine, tyrosine, valine, isoleucine, leucine, proline, histidine, lysine, arginine, and phenylalanine. Drawert and Reuther (1963) studied phenol as a humification inhibitor in protein hydrolysis. Finely ground 50-mg samples of grape leaves and vine were heated for five minutes at 50-60°C in the presence of 4 ml of 80% phenol. The mixture was then hydrolyzed in a sealed tube with 6 ml of 10 N hydrochloric acid for 20 hours at 105°C. The resulting solution was extracted with ether. The ether-phenol solution was extracted with water and the analysis was made on the water soluble fraction. The amino acid values obtained with the use of phenol as an inhibitor were considerably higher than those obtained without the additive.

Limited data have been reported on the effect of acid concentration on the hydrolysis of protein. The chromatographic analysis of Hepburn and Bradley (1965) showed no difference in the use of 4 N and 6 N hydrochloric acid in the hydrolysis of wheat. These results also agreed well with those of microbiological methods. Chromatographic analysis of 24-hour hydrolyzates resulted in lower serine values than those found by microbiological assay. Serine values approached those obtained by microbiological assay when the samples were hydrolyzed for eight hours with

4 N hydrochloric acid. However, the values for most of the other amino acids were lower due to incomplete hydrolysis.

Dye Absorption Determination of Protein Content of Wheat and Wheat Flour

The use of dye binding techniques for rapid quantitative estimation of protein has attracted considerable attention. Udy (1954, 1956) estimated the total protein content of wheat and wheat flour by measuring the concentration of unbound orange G dye colorimetrically at 485 mμ. It has been reported that basic groups on the protein molecule react with the dissociated sulfonic acid groups of orange G dye, at a low pH, to form an insoluble protein-dye complex. The correlation coefficient for the regression of percent protein and the mg of dye bound per gram of ground whole wheat was 0.992. The standard error of estimate was 0.22%. Likewise for wheat flour $r = 0.997$ and the standard error of estimate was 0.20%. The regression line was found not to pass through the origin. This suggested that constituents other than protein were binding dye. Further studies indicated starch would bind approximately 4.6 mg of dye per gram at pH 2.2. Fraenkel-Conrat, et al., (1944) reported analytical methods for the determination of the total acid and basic groups of proteins based upon the ability to combine with dyes in buffered alkaline or acid solutions. These authors concluded the number of basic groups binding orange G at pH 2.2 represented the sum of the guanidyl, imidazole, and free amino (α- and ϵ-) groups of proteins. Earlier work by Rawlins, et al., (1927), and Rawlins and Schmidt (1929, 1930) indicated the capacity of proteins to combine with dye could be correlated with the content of the free basic groups of arginine, lysine, and histidine.

MATERIALS AND METHODS

Kjeldahl Protein Determination

Wheat and Flour Samples. Hard Red Winter and Northern Spring wheat samples were composited by variety from equal portions of wheat from the 1963 crop. Samples of Pawnee (11669), Comanche (11673), Qv-Tm x Mql-Oro (12995), Chiefkan x Tenmarq (501097), and Chiefkan x Tenmarq (501099) were from Clovis, New Mexico; Lincoln and North Platte, Nebraska; Fort Collins, Colorado; Stillwater, Cherokee, Goodwell, and Woodard, Oklahoma; Bushland and Chillicothe, Texas; and Garden City, Hays, Colby and Manhattan, Kansas. Yogo (8033) and Warrior (13190) were from Hymore and Brookings, South Dakota, St. Paul and Crookston, Minnesota; and Huntley, Bozeman, Havre, and Moccasin, Montana. Karmont (6700) was from Huntley, Bozeman, Sidney and Havre, Montana. Thatcher (10003), Selkirk (13100), Marquis (2641), Lee (12488), and Pilot (11428) were from Brookings, Eureka, Hymore and Newell, South Dakota; Dickenson, Fargo, Minot and Williston, North Dakota; Morris and Crookston, Minnesota; and Havre, Huntley and Bozeman, Montana. Single samples of Soft Red Winter (Seneca, 12529) Durum (Wells, 13333, reg. 403) and White Club wheat (Omar, 13072, reg. 377) were obtained from agricultural experiment stations in Wooster, Ohio, Fargo, North Dakota, and Pullman, Washington, respectively.

Analytical determinations of wheat were made on samples ground on a Micro-Wiley mill to pass a 40-mesh sieve. Straight grade flour samples were obtained by milling the wheat on a Miag "Multimat" experimental mill.

Kjeldahl protein determinations were made on the wheat and flour samples according to AACC methods (1962).

Dye Absorption for Protein Determination

Dye absorption was determined using dye and equipment obtained from the Udy Analyzer Company. The reagent dye used was acid orange 12 in a phosphate buffered system at pH 1.7. One gram samples of flour and 0.8 gram samples of wheat were weighed and placed in a reaction chamber with 40 ml of the reagent dye solution. The mixture was reacted for three minutes with vigorous shaking. The mixture was then transferred to a squeeze-type polyethylene bottle fitted with a fiberglass filter disc in the dropper cap. Light transmittance through the resulting dye solution was read on the colorimeter as the filtrate was transferred dropwise into the flow-through cuvette. The colorimeter was previously calibrated to 32% transmittance with a reference dye solution.

Amino Acid Analysis

Acid hydrolysis was used in the preparation of samples for amino acid analysis, Private Communication (1963). In this procedure test tubes were narrowed after the addition of 100 mg of sample. Then 6 N hydrochloric acid was added at the rate of approximately 1 ml per 10 mg of protein. A vacuum was pulled on the tubes which were in a dry-ice alcohol bath. The tubes were sealed while under the vacuum. Contents of the tubes were hydrolyzed at 110°C for 22 hours and filtered through a fritted disc funnel to remove the insoluble humin.

The filtrate was evaporated to dryness three times under partial vacuum and then diluted to 10 ml with 0.2 N sodium citrate buffer, pH 2.2. All hydrolyzates were stored at -20°C until analyzed. The hydrolyzates were carmel-colored indicating the presence of a small amount of humin material. Amino acid analyses of the hydrolyzates were made following the procedure of Spackman, et al., (1958) and Moore, et al., (1958) using a Beckman Model 120B Amino Acid Analyzer.

RESULTS AND DISCUSSION

Comparisons of Varieties

The amino acid composition of the eight composites of Hard Red Winter wheats and their straight grade flours are summarized in Tables 1 and 2. Values are reported as grams of amino acid per 100 grams of protein calculated from the amino acid analyzer.[1] The small differences found among the eight varieties might be caused by varietal effects or might be due to the different levels of protein content between varieties. Hepburn and Bradley (1965) observed that the concentrations of several amino acids changed with a change in the protein level.

The amino acid composition of the five wheat composites of Northern Spring varieties and the composites of Soft Red Winter, White Club and Durum classes is reported in Table 3. The composition of the straight grade flours from these wheats is summarized in Table 4. These values

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[1] Protein is the total grams of amino acids and ammonia accounted for by the amino acid analyzer.

Table 1. Amino acid composition of Hard Red Winter wheat (grams of amino acid per 100 grams protein*).

Code Number**	281	282	283	284	285	286	287	288	Average
Protein***	14.74	15.95	14.80	15.03	16.10	12.65	14.84	16.87	15.12
Lysine	2.95	2.72	2.86	2.65	2.67	2.94	2.87	2.99	2.83
Histidine	2.54	2.38	2.34	2.37	2.37	2.38	2.43	2.33	2.39
Ammonia	3.31	3.40	3.58	3.60	3.73	3.52	3.78	3.82	3.59
Arginine	4.69	4.21	4.54	4.34	4.26	4.35	4.49	4.30	4.40
Aspartic Acid	5.07	3.82	4.92	4.98	4.98	5.02	4.90	4.77	4.93
Threonine	2.86	2.73	2.75	2.72	2.72	3.04	2.77	2.63	2.78
Serine	4.59	4.50	4.60	4.49	4.46	4.67	4.51	4.46	4.54
Glutamic Acid	31.37	33.14	32.48	32.85	32.57	30.30	32.49	33.02	32.28
Proline	10.13	10.53	10.34	10.65	10.59	10.37	10.70	11.18	10.56
Glycine	4.09	4.08	4.05	3.97	3.96	4.27	3.97	3.85	4.03
Alanine	3.61	3.44	3.43	3.48	3.90	3.77	3.41	3.31	3.54
Half Cystine	2.65	2.23	2.53	2.43	2.23	2.84	2.40	2.11	2.43
Valine	4.39	4.20	4.16	4.14	4.23	4.53	4.17	4.05	4.23
Methionine	1.29	1.16	1.22	1.20	1.12	1.37	1.16	1.07	1.20
Isoleucine	3.52	3.42	3.34	3.33	3.41	3.53	3.43	3.42	3.42
Leucine	6.79	6.68	6.60	6.50	6.60	6.80	6.64	6.56	6.65
Tyrosine	1.73	1.62	1.77	1.70	1.63	1.73	1.57	1.49	1.66
Phenylalanine	4.46	4.76	4.55	4.65	4.64	4.63	4.37	4.67	4.59

*Protein content was obtained by adding the grams of amino acids and ammonia accounted for by the amino acid analyzer.

**Varieties:
281 - Pawnee (11669)
282 - Comanche (11673)
283 - Qv - Tm x Mql-Oro (12995)
284 - Chiefkan x Tenmarq (501097)
285 - Chiefkan x Tenmarq (501099)
286 - Yogo (8033)
287 - Warrior (13190)
288 - Karmont (6700)

***%, Kjeldahl protein N X 5.70 (moisture-free basis)

Table 2. Amino acid composition of Hard Red Winter wheat flour (grams of amino acid per 100 grams protein*).

Code Number**	281	282	283	284	285	286	287	288	Average
Protein***	14.20	15.00	13.96	14.45	15.21	12.07	14.38	15.70	14.37
Lysine	2.00	1.90	2.02	1.93	2.23	2.06	2.08	1.98	2.02
Histidine	2.05	2.04	2.01	2.09	2.45	2.04	2.06	2.02	2.10
Ammonia	3.89	3.89	3.99	4.08	4.66	3.69	3.72	3.83	3.97
Arginine	3.37	3.00	3.28	3.11	3.58	3.18	3.17	3.09	3.22
Aspartic Acid	3.87	3.66	3.83	3.80	3.76	3.88	3.88	3.72	3.80
Threonine	2.59	2.53	2.62	2.50	2.47	2.83	2.61	2.51	2.58
Serine	4.53	4.61	4.77	4.52	4.33	4.57	4.50	4.51	4.54
Glutamic Acid	35.64	36.03	35.36	35.70	35.04	34.60	34.78	36.24	35.42
Proline	11.14	11.82	11.70	11.80	11.74	11.61	11.45	12.18	11.68
Glycine	3.40	3.42	3.56	3.33	3.26	3.44	3.49	3.29	3.40
Alanine	2.97	2.83	2.87	2.88	2.85	3.06	3.00	2.81	2.91
Half Cystine	2.65	2.38	2.39	2.46	2.23	2.74	3.25	2.15	2.53
Valine	4.09	4.03	3.97	4.04	3.96	4.36	4.23	3.97	4.08
Methionine	1.29	1.18	1.21	1.26	1.13	1.35	1.24	1.16	1.23
Isoleucine	3.54	3.55	3.53	3.55	3.54	3.68	3.66	3.56	3.58
Leucine	6.75	6.66	6.71	6.80	6.57	6.93	6.75	6.67	6.73
Tyrosine	1.57	1.45	1.36	1.30	1.38	1.29	1.45	1.31	1.39
Phenylalanine	4.65	5.01	4.83	4.86	4.81	4.69	4.69	4.98	4.82

*Protein content was obtained by adding the grams of amino acids and ammonia accounted for by the amino acid analyzer.

**Varieties:
281 - Pawnee (11669)
282 - Comanche (11673)
283 - Qv - Tm x Mql-Oro (12995)
284 - Chiefkan x Tenmarq (501097)
285 - Chiefkan x Tenmarq (501099)
286 - Yogo (8033)
287 - Warrior (13190)
288 - Karmont (6700)

***%, Kjeldahl Protein N X 5.70 (moisture free basis)

Table 3. Amino acid composition of Northern Spring, Soft Red Winter, Durum, and White Club wheats (grams of amino acid per 100 grams of protein*).

	NORTHERN SPRING					Average	SRW	Durum	Club
Code Number**	289	290	291	292	293	NS	294	295	296
Protein***	16.95	17.17	16.07	16.97	15.85	16.60	15.87	14.20	8.89
Lysine	2.60	2.79	2.62	2.51	2.69	2.64	2.72	2.62	3.22
Histidine	2.26	2.29	2.25	2.28	2.30	2.28	2.34	2.19	2.39
Ammonia	3.65	3.77	3.44	3.61	3.42	3.58	3.50	3.60	3.09
Arginine	4.09	4.09	4.28	4.33	4.34	4.23	4.22	4.27	4.66
Aspartic Acid	4.94	4.79	4.96	4.98	5.20	4.97	4.89	5.09	5.85
Threonine	2.75	2.66	2.90	2.77	2.94	2.80	2.83	2.76	3.12
Serine	4.55	4.53	4.61	4.56	4.59	4.57	4.55	4.49	4.71
Glutamic Acid	32.30	32.85	31.47	31.99	31.39	32.00	32.30	32.22	29.01
Proline	11.10	10.84	10.56	10.70	10.53	10.75	10.87	10.34	9.93
Glycine	4.07	3.84	4.10	3.91	4.11	4.01	3.92	3.69	4.37
Alanine	3.52	3.37	3.63	3.55	3.64	3.54	3.51	3.59	4.19
Half Cystine	2.13	2.58	2.82	2.61	2.80	2.59	2.17	2.26	2.21
Valine	4.27	4.25	4.41	4.36	4.45	4.35	4.32	4.32	4.84
Methionine	.95	1.16	1.22	1.24	1.18	1.15	1.12	1.27	1.30
Isoleucine	3.50	3.84	3.50	3.55	3.54	3.59	3.57	3.96	3.66
Leucine	6.72	6.68	6.74	6.79	6.77	6.74	6.78	6.75	7.05
Tyrosine	1.74	1.68	1.90	1.71	1.55	1.72	1.57	1.82	1.70
Phenylalanine	4.90	4.05	4.64	4.61	4.61	4.56	4.87	4.80	4.75

*Protein content was obtained by adding the grams of amino acids and ammonia accounted for by the amino acid analyzer.

**Varieties:

289 - Thatcher (10003)
290 - Selkirk (13100)
291 - Marquis (2641)
292 - Lee (12488)
293 - Pilot (11428)
294 - Seneca (12529)
295 - Wells (13333, reg. 403)
296 - White Club Wheat Omar (13072, reg. 377)

***%, Kjeldahl protein N X 5.70 (moisture free basis)

Table 4. Amino acid composition of Northern Spring, Soft Red Winter, Durum, and White Club wheat flours (grams of amino acid per 100 grams protein*).

	NORTHERN SPRING					Average	SRW	Durum	Club
Code Number**	289	290	291	292	293	NS	294	295	296
Protein***	15.75	15.61	14.83	16.25	14.70	15.43	13.03	13.21	7.52
Lysine	1.85	1.73	1.87	1.74	1.67	1.77	2.35	2.03	2.65
Histidine	2.09	1.99	2.04	1.97	1.85	1.99	3.55	2.13	2.39
Ammonia	4.00	4.11	4.03	3.90	3.59	3.93	4.59	4.07	3.98
Arginine	3.08	3.00	3.14	3.03	2.87	3.02	3.73	3.39	3.69
Aspartic Acid	3.84	3.63	3.75	3.74	3.83	3.76	3.66	4.25	4.35
Threonine	2.41	2.43	2.53	2.45	2.61	2.48	2.47	2.52	2.71
Serine	4.31	4.47	4.46	4.47	4.61	4.46	4.35	4.37	4.66
Glutamic Acid	36.37	36.25	35.45	36.06	35.77	35.98	35.16	34.98	32.06
Proline	12.07	11.82	11.65	11.40	11.63	11.71	11.36	11.25	10.93
Glycine	3.29	3.14	3.39	3.19	3.29	3.26	3.16	3.10	3.53
Alanine	2.94	2.91	3.07	3.01	3.09	3.00	2.98	3.22	3.34
Half Cystine	2.38	2.56	2.71	2.66	2.64	2.59	2.39	2.33	2.94
Valine	3.84	3.89	4.11	4.03	4.11	4.00	3.87	4.03	4.35
Methionine	1.24	1.23	1.30	1.29	1.39	1.29	1.24	1.31	1.47
Isoleucine	3.43	3.48	3.57	4.26	3.67	3.68	3.44	3.67	3.65
Leucine	6.53	6.61	6.75	6.73	6.90	6.70	6.48	6.72	6.99
Tyrosine	1.45	1.76	1.42	1.39	1.62	1.53	1.45	1.74	1.44
Phenylalanine	4.87	5.00	4.76	4.66	4.87	4.83	4.78	4.89	4.87

*Protein content was obtained by adding the grams of amino acids and ammonia accounted for by the amino acid analyzer.

**Varieties:

289 - Thatcher (10003)
290 - Selkirk (13100)
291 - Marquis (2641)
292 - Lee (12488)
293 - Pilot (11428)
294 - Seneca (12529)
295 - Wells (13333, reg. 403)
296 - White Club Wheat Omar (13072, reg. 377)

***%, Kjeldahl protein N X 5.70 (moisture free basis)

are reported on the same basis as those in Tables 1 and 2. Only small differences were found when comparing the Northern Spring varieties, Soft Red Winter, White Club and Durum wheats.

Comparisons between classes of wheat should be made with extreme caution because of the differing levels of total protein. Amino acid distribution has been found to change with total protein as is demonstrated in this research and by the work of others. Thus in comparing classes with different levels of protein, one would be unable to distinguish between variations due to class of wheat and variations caused by the different protein levels.

Duplicate analyses of amino acids were made for only the 16 samples of whole wheat. The values in Tables 1 and 3 are reported as averages of these duplicate analyses. The accuracy of the analysis is indicated in Table 5 which lists the average difference between duplicate analyses. This shows the results of taking the difference between duplicate analyses for each of the 16 composites and computing an average of these differences. Also listed is the largest difference between duplicate analyses for the respective amino acids. It should be noted the average differences are considerably less, in all cases, than the largest difference between the duplicates.

Nitrogen Recovery in Amino Acid Analysis

The nitrogen from amino acids and ammonia was calculated as a percentage of Kjeldahl nitrogen (Table 6). For the 16 whole wheat composites an average of 91% of the Kjeldahl nitrogen was accounted for with a range from 86.3 to 95.8 percent. From the analysis of the flour samples

Table 5. The average and largest difference between duplicate analyses for 16 samples.

Amino Acid	Largest Difference*	Average Difference*
Lysine	0.33	0.14
Histidine	0.26	0.12
Arginine	0.68	0.25
Aspartic Acid	0.37	0.09
Threonine	0.23	0.06
Serine	0.21	0.08
Glutamic Acid	2.00	0.47
Proline	0.50	0.20
Glycine	0.34	0.09
Alanine	0.30	0.09
Half Cystine	0.52	0.23
Valine	0.59	0.15
Methionine	0.38	0.09
Isoleucine	0.64	0.14
Leucine	0.23	0.09
Tyrosine	0.34	0.17
Phenylalanine	0.52	0.17

*Grams of amino acid per 100 grams protein

the nitrogen which was accounted for averaged 95.6% with a range from 90.8 to 104.4 percent. These amounts of nitrogen accounted for are in the same range as reported by Hepburn and Bradley (1965).

Comparison of Amino Acid Concentration of Wheat and its Flour

The amino acid composition of wheat and flour protein has been compared by several workers. Barton-Wright, et al., (1946), Hepburn, et al., (1957, 1960), Horn, et al., (1958) and Pence, et al., (1964) have reported the effects of milling wheat into flour. In the present study the differences found after milling Hard Red Winter and Northern

Table 6. Nitrogen accounted for in wheat and wheat flour samples.

Sample Number	Nitrogen[1] Accounted For (Wheat)	Nitrogen[1] Accounted For (Flour)
	%	%
1	90.3	95.1
2	88.3	95.8
3	89.5	93.3
4	91.9	96.5
5	90.1	99.7
6	90.9	90.8
7	91.0	93.7
8	88.8	93.7
9	95.8	92.8
10	92.4	95.2
11	92.2	94.9
12	94.4	93.2
13	91.8	104.4
14	90.3	96.2
15	91.6	92.7
16	86.3	99.9
Overall Average	91.0	95.6

$$^{1}\text{Nitrogen Accounted For} = \frac{\text{Nitrogen from the Amino Acids and Ammonia}}{\text{Kjeldahl Nitrogen}} \times 100$$

Spring wheats into flour are shown in Figs. 1 and 2 respectively. The graphs indicate differences in the amounts of amino acid in the proteins of a wheat and its flour. Similar trends in amino acid composition were found in both Hard Red Winter and Northern Spring classes of wheat. Milling the Hard Red Winter wheat into flour resulted in a decrease in amino acid content of the protein as indicated: lysine 29%, histidine 12%, arginine 27%, aspartic acid 22%, threonine 7%, glycine 16%, alanine 18%, valine 4%, and tyrosine 16%. Amino acids which were more concentrated in the flour protein were glutamic acid (10%), proline (10%), half cystine (4%), isoleucine (5%), and phenylalanine (5%). These data are similar

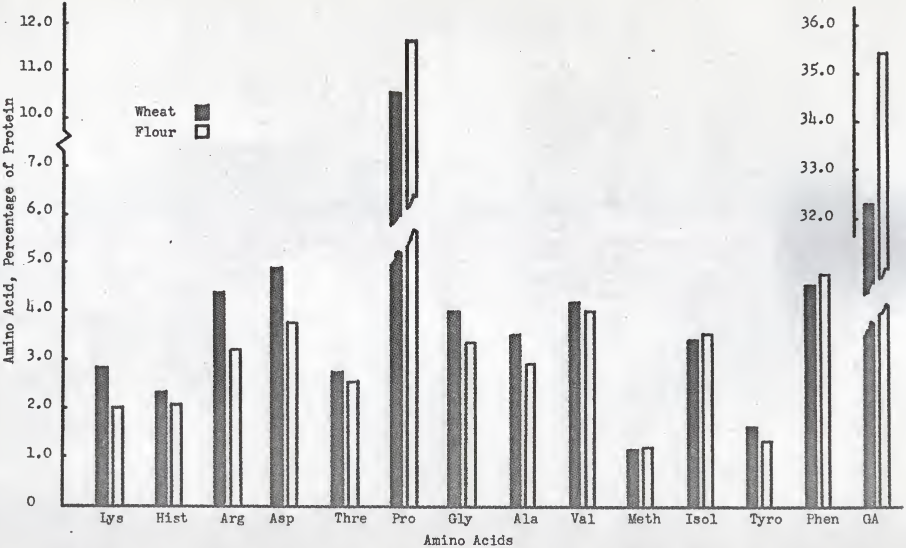

Fig. 1. Relationship of wheat to flour with respect to the amino acid distribution in the protein of Hard Red Winter wheat (average of 8 varieties).

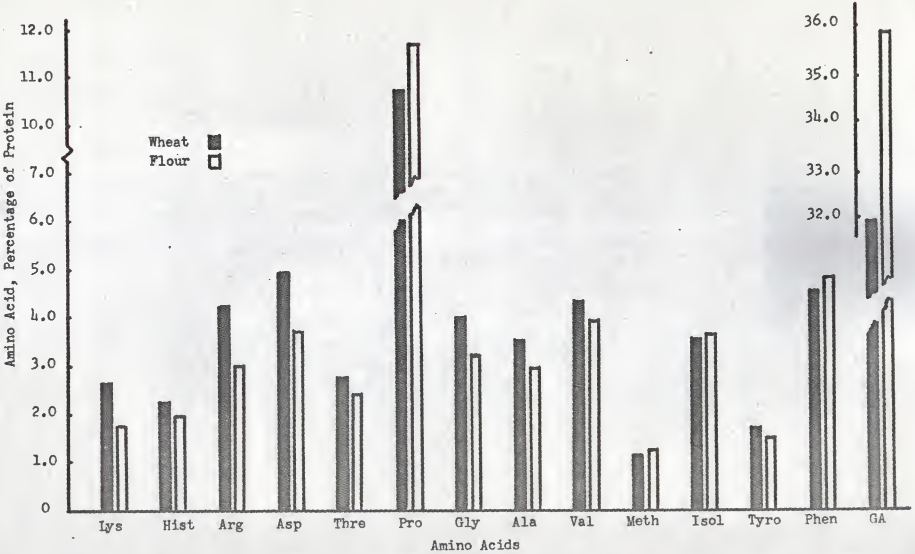

Fig. 2. Relationship of wheat to flour with respect to the amino acid distribution in the protein of Northern Spring wheat (average of 5 varieties).

to those reported in the literature and indicate to some extent the distribution of amino acids in the wheat kernel. The data indicate that glutamic acid, proline, isoleucine, half cystine, and phenylalanine are higher in the protein of the starchy endosperm portion than in the whole wheat. Milling of Northern Spring wheat into flour lowered the concentration of the following amino acids: lysine 33%, histidine 13%, arginine 29%, aspartic acid 24%, threonine 11%, glycine 19%, alanine 15%, valine 8%, and tyrosine 11%. The amino acids that were more concentrated in the flour than in the wheat protein are: proline 10%, glutamic acid 12%, methionine 12%, and phenylalanine 6%. Hepburn, et al., (1957) showed similar trends when comparing the wheat and flour of two commercial blends of Hard Red Spring wheat and two blends of Hard Red Winter wheat.

Statistical analyses were made by use of regression and correlation procedures according to Snedecor (1956). All correlations computed were based on the data for whole wheat of the various varieties. All protein values are corrected to zero moisture content unless otherwise specified.

A regression line and correlation coefficient was calculated for the relationship of Kjeldahl protein content (not corrected for moisture) and percent transmittance of the unbound dye. The statistical analysis was made using data for all 16 varieties of wheat. The equation [2] for the regression line was $Y = 0.49\ X + 3.66$ with a standard deviation of 0.429 about the line. The standard deviation of the slope was 0.030.

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[2] In this equation Y represents Kjeldahl protein content and X represents percent transmittance.

A highly significant correlation ($p < .01$) was found between Kjeldahl protein and percent transmittance.

A significant negative correlation at the 1% level was also found between Kjeldahl protein content and the sum of the basic amino acids lysine, histidine, and arginine as percentage of the protein.[3] All 16 varieties were used in this regression with the intention of determining the general effect that increasing protein content has on the concentration of basic amino acids in the protein. The equation[4] for the regression line was $Y = 11.44 - 0.13\ X$ with a standard deviation of 0.286 about the line. The slope had a standard deviation of 0.035.

The samples were then considered as two sets of data. This division into the two classes of wheat was made with the intention of removing any effect the class might have on a linear trend determined by regression analysis. One set of samples consisted of the eight Hard Red Winter wheats with an average Kjeldahl protein of 15.12% and a range from 12.65% to 16.87%. The second group included the five Northern Spring wheats comprising a 16.60% Kjeldahl protein average with a range from 15.85% to 17.17%.

A separate regression analysis was made for both Hard Red Winter and Northern Spring wheat. Comparisons made were between Kjeldahl

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[3] $$\text{Basic amino acids as percentage of protein} = \frac{\text{Summation of the grams of lysine, histidine and arginine}}{\text{Total grams of amino acids and ammonia accounted for by the amino acid analyzer}} \times 100$$

[4] In this equation Y represents the sum of the basic amino acids and X represents Kjeldahl protein content.

protein content (not corrected for moisture) and percent transmittance. No significant correlation was found between the protein content and percent transmittance for the Northern Spring wheats. However, a highly significant correlation ($p < .01$) was observed when this relationship was considered using the Hard Red Winter wheats. The equation[5] $Y = 0.53 X + 5.05$ for the regression line was obtained for this relationship. As stated before a highly significant correlation coefficient 0.988 existed. The standard deviation about the regression line was 0.175 and the standard deviation of the slope was 0.032.

Regressions and Correlations of Amino Acids with Kjeldahl Protein

The samples were again considered as two classes of wheat. The relationship of the sum of the basic amino acids as a percentage of protein[6] and Kjeldahl protein content was observed. No significant correlation was found for this relationship when the data from both the Hard Red Winter and Northern Spring wheats were used.

Each of the 17 amino acids was also considered as a function of the Kjeldahl protein content. Correlation coefficients were calculated for each and a regression equation obtained for those which were

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[5] In this equation Y represents Kjeldahl protein content and X represents percent transmission.

[6]

$$\text{Basic amino acids as percentage of protein} = \frac{\text{Summation of the grams of lysine, histidine and arginine}}{\text{Total grams of amino acids and ammonia accounted for by the amino acid analyzer}} \times 100$$

significant. The unit of amino acid used in the computation of all subsequent correlations is expressed as the percentage of the protein.[7]

The data for those amino acids represented in Table 7 were significantly correlated with the Kjeldahl protein content. These correlations were calculated from the data of eight Hard Red Winter wheat composites.

It is interesting that glutamic acid was the only amino acid which was correlated positively with Kjeldahl protein. This indicates the concentration of glutamic acid in protein increases as the protein level increases. The slope of the trend line was also found to be larger as compared to those of the other amino acids which indicates a larger change in concentration with a change in protein level. The data from the amino acids shown were highly significant ($p < .01$) except for valine which was significant at the 5% level.

The data from the five Northern Spring wheats were treated statistically in the same manner as those from the Hard Red Winter wheats. Significant results are shown in Table 8.

In Northern Spring wheat glutamic acid was again found to be positively correlated with Kjeldahl protein content. The amounts of the amino acids shown in Table 8 were significantly correlated ($p < .05$) except for threonine which was significantly correlated at the 1% level. Glycine, half cystine and methionine were correlated with Kjeldahl protein in the Hard Red Winter but not in the Northern Spring wheat.

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[7] $$\text{Amino acid, percentage of protein} = \frac{\text{Grams of the amino acid}}{\text{Total grams of amino acids and ammonia accounted for by the amino acid analyzer}} \times 100$$

Table 7. The relationships between Kjeldahl protein content and the percentage of amino acid in the protein[1] of Hard Red Winter wheat.

Amino Acid	Correlation	Level of Significance[2]	Equation of Regression Line[3]	$S_{X \cdot Y}$[4]	S_b[5]
		%			
Valine	-0.806	5	Y = 5.72 - 0.10 X	0.098	0.029
Threonine	-0.931	1	Y = 4.16 - 0.09 X	0.048	0.014
Serine	-0.888	1	Y = 5.35 - 0.05 X	0.037	0.011
Glutamic Acid	+0.862	1	Y =22.30 + 0.66 X	0.528	0.158
Glycine	-0.856	1	Y = 5.31 - 0.08 X	0.069	0.020
Half Cystine	-0.955	1	Y = 5.20 - 0.18 X	0.077	0.023
Methionine	-0.934	1	Y = 2.27 - 0.07 X	0.036	0.011

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[1]

$$\text{Amino acid, percentage of protein} = \frac{\text{Grams of the amino acid}}{\text{Total grams of amino acids and ammonia accounted for by the amino acid analyzer}} \times 100$$

[2] The correlation coefficients at the 5% and 1% levels of significance are 0.707 and 0.834 respectively.

[3] The percentage of amino acid in the protein as represented by Y and X represents Kjeldahl protein content.

[4] Represents the standard deviation of the Y's in the population about the trend line.

[5] Represents the standard deviation of the slope of the linear trend line.

Table 8. The relationships between Kjeldahl protein content and the percentage of amino acid in the protein[1] of Northern Spring wheat.

Amino Acid	Correlation	Level of Significance[2]	Equation of Regression Line[3]	$S_{X \cdot Y}$[4]	S_b[5]
		%			
Threonine	-0.973	1	$Y = 5.90 - 0.19\,X$	0.030	0.025
Serine	-0.912	5	$Y = 5.38 - 0.05\,X$	0.015	0.012
Glutamic Acid	+0.915	5	$Y = 16.60 + 0.93\,X$	0.281	0.235
Valine	-0.907	5	$Y = 6.54 - 0.13\,X$	0.042	0.035

[1] $$\text{Amino acid, percentage of protein} = \frac{\text{Grams of the amino acid}}{\text{Total grams of amino acids and ammonia accounted for by the amino acid analyzer}} \times 100$$

[2] The correlation coefficients at the 5% and 1% levels of significance are 0.878 and 0.959 respectively.

[3] The percentage of amino acid in the protein is represented by Y and X represents Kjeldahl protein content.

[4] Represents the standard deviation of the Y's in the population about the trend line.

[5] Represents the standard deviation of the slope of the linear trend line.

Regressions and Correlations of Amino Acids with Dye Absorption

Rawlins, et al., (1927) and Fraenkel-Conrat (1944) indicated that dye binding might be used to determine the total amount of basic amino acids present in proteins. They suggested the sulfonic acid groups of orange G dye would bind with the guanidine, imidazole, and free amino groups of the protein. The protein sources used by these workers were not cereal grains.

The relationship between percent transmission of the unbound dye (acid orange 12) and the sum of the basic amino acids[8] (lysine, histidine, and arginine) was found to be nonsignificant in these studies. These correlations were calculated for both the Hard Red Winter and Northern Spring classes of wheat.

Calculations were also made to determine possible correlations between the dye absorbed and the individual amino acids. Shown in Table 9 are the significant correlations found when the data of the eight Hard Red Winter composites were used.

The data of all the amino acids presented in Table 9 are negatively correlated with dye absorption except for glutamic acid and proline. The slopes are relatively small indicating that a small change in the amino acid concentration accompanies a large change in the dye absorbed.

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[8] Basic amino acids as percentage of protein $= \frac{\text{Summation of the grams of lysine, histidine and arginine}}{\text{Total grams of amino acids and ammonia accounted for by the amino acid analyzer}} \times 100$

Table 9. The relationships between percent transmittance and the percentage of amino acid in the protein[1] of Hard Red Winter wheat.

Amino Acid	Correlation	Level of Significance[2]	Equation of Regression Line[3]	$S_{X \cdot Y}$[4]	S_b[5]
		%			
Threonine	-0.931	1	$Y = 4.77 - 0.06\ X$	0.048	0.009
Serine	-0.924	1	$Y = 5.76 - 0.04\ X$	0.031	0.005
Glutamic Acid	+0.847	1	$Y = 18.08 + 0.41\ X$	0.552	0.103
Glycine	-0.894	1	$Y = 5.96 - 0.06\ X$	0.060	0.011
Half Cystine	-0.955	1	$Y = 6.43 - 0.11\ X$	0.077	0.014
Valine	-0.806	5	$Y = 6.38 - 0.06\ X$	0.098	0.018
Methionine	-0.948	1	$Y = 2.77 - 0.04\ X$	0.032	0.006

[1] $$\text{Amino acid, percentage of protein} = \frac{\text{Grams of the amino acid}}{\text{Total grams of amino acids and ammonia accounted for by the amino acid analyzer}} \times 100$$

[2] The correlation coefficients at the 5% and 1% levels of significance are 0.707 and 0.834 respectively.

[3] The percentage of the amino acids in the protein are represented by Y and X represents the percent transmission of the unbound dye.

[4] Represents the standard deviation of the Y's in the population about the trend line.

[5] Represents the standard deviation of the slope of the linear trend line.

It is interesting that none of the basic amino acids was significantly correlated with the amount of dye absorbed. When correlations of individual amino acids were made with percent transmittance for Northern Spring wheat, none were significantly correlated.

SUMMARY AND DISCUSSION

Samples representing eight varieties of Hard Red Winter wheat, five varieties of Northern Spring and single varieties of Soft Red Winter, White Club, and Durum wheats showed only small differences when amino acid levels were compared. Distinct differences in the amino acid content of the protein were noted between wheat and its flour. The milling of wheat into flour significantly lowered the concentrations of lysine, histidine, arginine, aspartic acid, threonine, glycine, alanine, valine, and tyrosine in both Hard Red Winter and Northern Spring wheat. In Hard Red Winter wheat the amino acids which were more concentrated in the flour than in the wheat included glutamic acid, proline, half cystine, isoleucine and phenylalanine. In Northern Spring wheat glutamic acid, proline, phenylalanine, and methionine were more concentrated in the flour than in the wheat.

Kjeldahl protein content was significantly correlated with the ability of the samples to bind acid orange 12 dye. Protein levels determined in this way were negatively correlated with the sum of the basic amino acids (lysine, histidine, and arginine) when data from all 16 varieties were used. However, when the samples were divided into Hard Red Winter wheat and Northern Spring wheat there failed to be a significant correlation between the sum of the basic amino acids and the amount of dye absorbed.

Amino acid data from the Hard Red Winter wheat were found to be correlated with Kjeldahl protein. Negative correlations were observed with threonine, serine, glycine, half cystine, and methionine. Glutamic acid, however, was found to be positively correlated with protein.

The amino acid concentrations in the winter wheat were also found to be related with dye binding capacities. The following amino acids were found to be negatively correlated with dye binding capacity as measured by the percent transmittance: threonine, serine, glycine, half cystine, valine, methionine, leucine, and tyrosine. Glutamic acid was found to be positively correlated with the amount of dye absorbed.

When the data for the basic amino acids were combined and correlated with the percent transmission of the dye, no significant correlations were noted for the winter and spring wheats.

Amino acid data from the Northern Spring wheat were correlated with Kjeldahl protein. When the correlations were calculated, threonine, serine, and valine were found to have significant negative correlations while glutamic acid had a significant positive correlation. Northern Spring wheat data were also related with percent transmittance and no correlations were significant.

SUGGESTIONS FOR FUTURE RESEARCH

The results of this preliminary study indicate dye binding techniques may be useful as a means of estimating the relative amino acid composition of protein. More research must be done in an attempt to determine reliable regression trends or relationships between the various amino acids and the ability of protein to bind dye. From these relationships one could, in table form, present the levels of the various amino acids with respect to the amount of dye absorbed. By use of the table and estimation of the amino acid concentration could be made.

Relationships should be observed between dye binding and various methods used to determine flour and baking quality of wheat. If significant correlations were found, perhaps the dye analysis would prove to be a rapid method for estimating quality traits.

Other studies should be conducted on hydrolysis procedures when working with large amounts of carbohydrate. Little work has been reported on hydrolyses carried out under these conditions. These conditions are common when preparing cereal hydrolyzates for ion exchange chromatography. Also, research should be conducted to determine the effect, if any, that carbohydrates have on the recovery of amino acids.

Genetic studies to magnify the small differences in amino acid composition within different varieties of wheat might be an extremely fruitful area of research. This should be done with the intention of increasing the amounts of nutritionally essential amino acids or increasing the concentration of other critical amino acids.

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APPENDIX

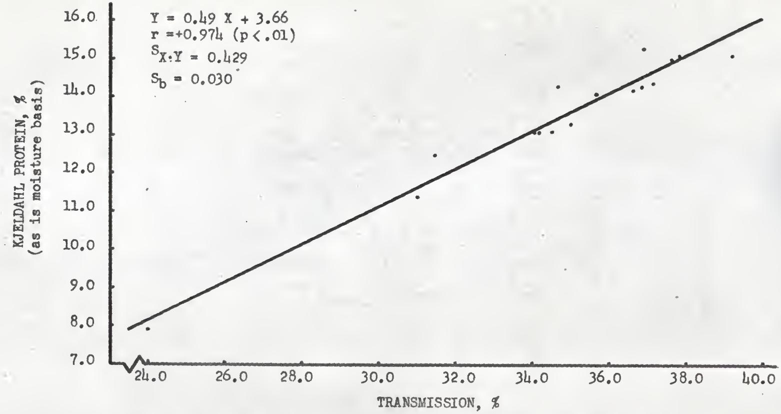

Fig. 3. The correlation between percent transmission and percent Kjeldahl protein content. (16 varieties of wheat)

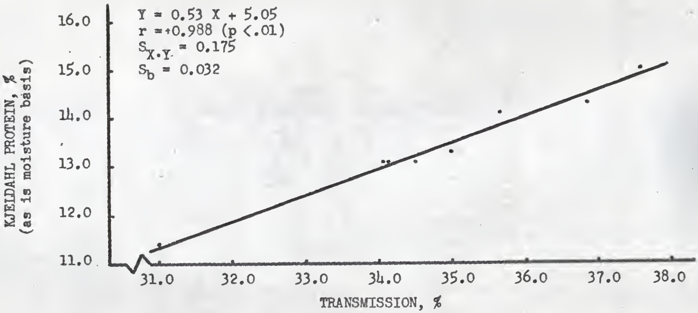

Fig. 4. The correlation between percent Kjeldahl protein content and percent transmission of the dye in Hard Red Winter wheat.

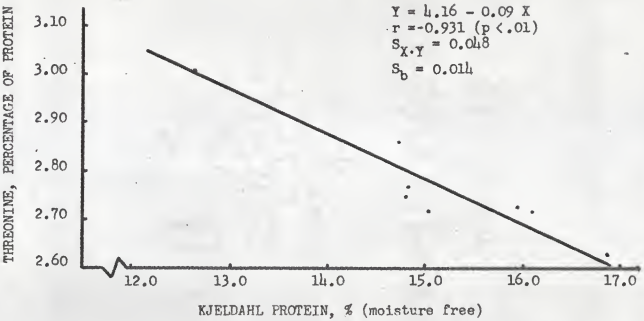

Fig. 5. The correlation between Kjeldahl protein content and the percent threonine in the protein of Hard Red Winter wheat.

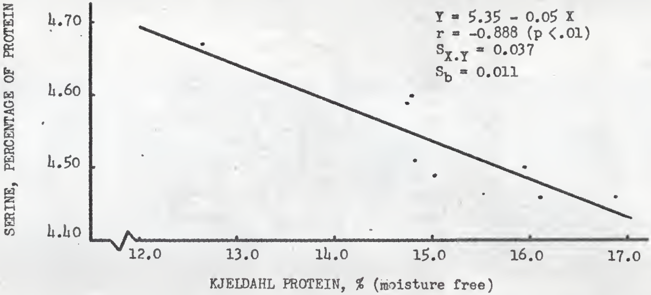

Fig. 6. The correlation between Kjeldahl protein content and the percent serine in protein of Hard Red Winter wheat.

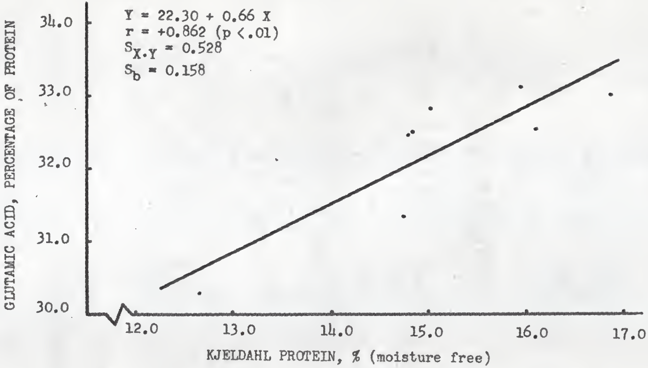

Fig. 7. The correlation between Kjeldahl protein content and the percent glutamic acid in protein of Hard Red Winter wheat.

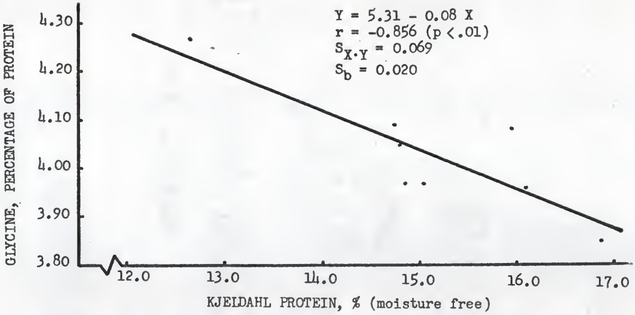

Fig. 8. The correlation between Kjeldahl protein content of the percent glycine in protein of Hard Red Winter wheat.

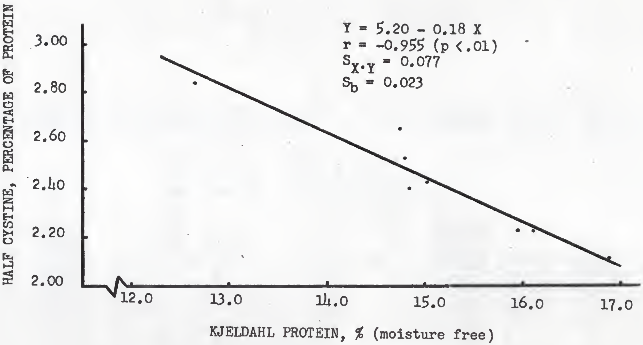

Fig. 9. The correlation between Kjeldahl protein content and the percent half cystine in protein of Hard Red Winter wheat.

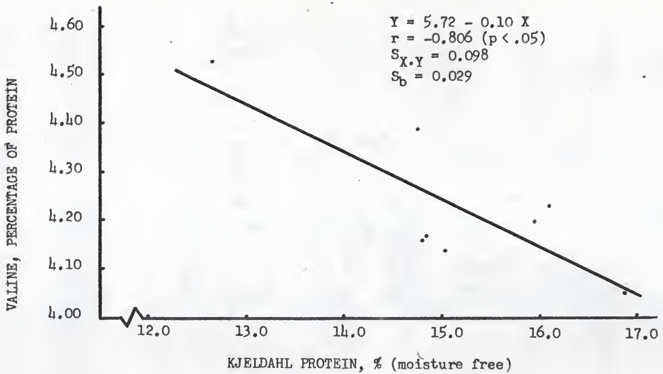

Fig. 10. The correlation between Kjeldahl protein content and the percent valine in protein of Hard Red Winter wheat.

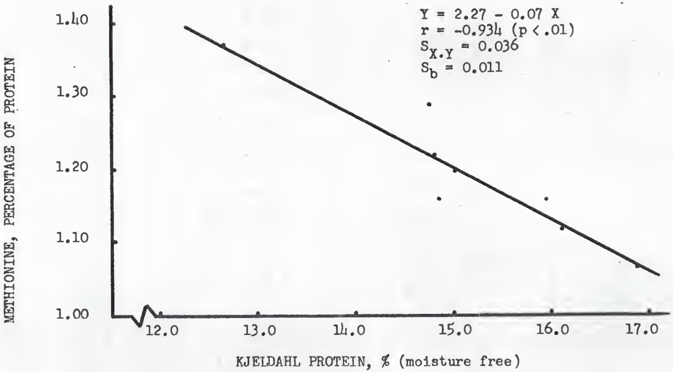

Fig. 11. The correlation between Kjeldahl protein content and the percent methionine in protein of Hard Red Winter wheat.

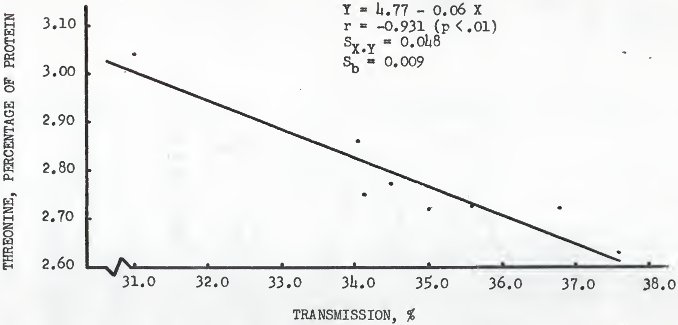

Fig. 12. The correlation between percent transmission and the percent threonine in the protein of Hard Red Winter wheat.

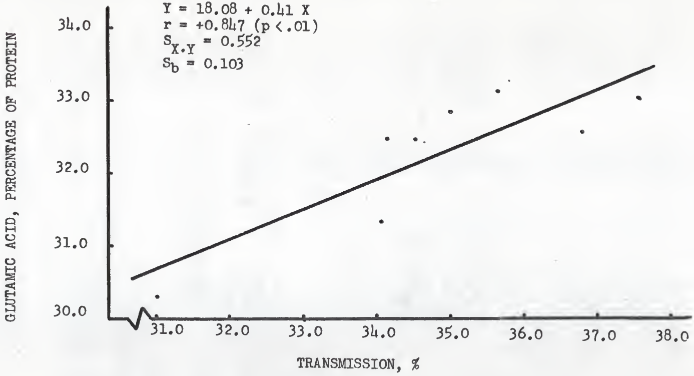

Fig. 13. The correlation between percent transmission and the percent glutamic acid in the protein of Hard Red Winter wheat.

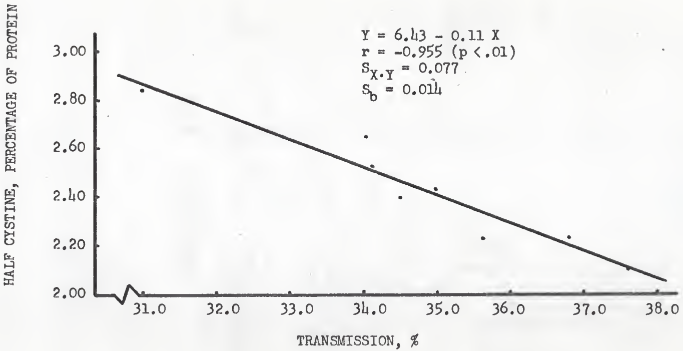

Fig. 14. The correlation between percent transmission and the percent half cystine in protein of Hard Red Winter wheat.

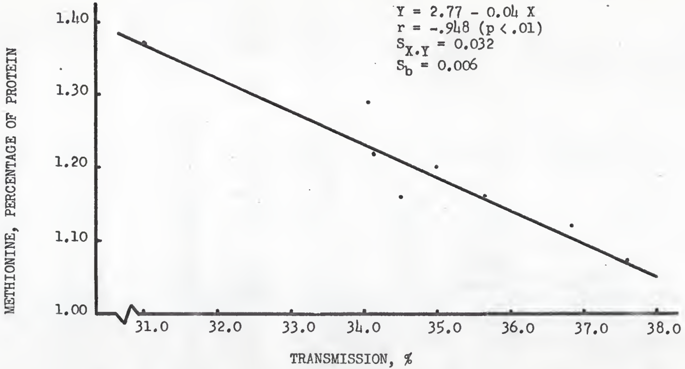

Fig. 15. The correlation between percent transmission and the percent methionine in protein of Hard Red Winter wheat.

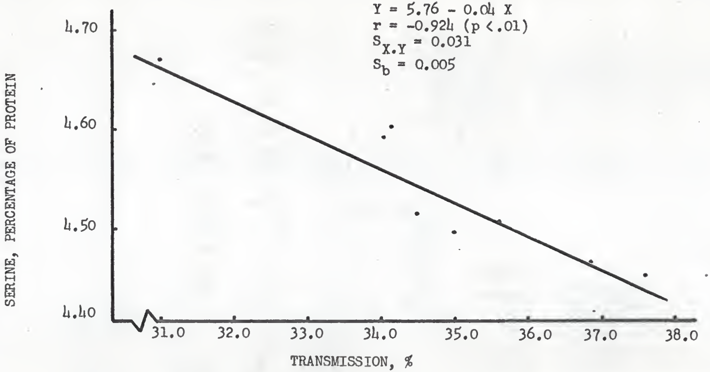

Fig. 16. The correlation between percent transmission and the percent serine in protein of Hard Red Winter wheat.

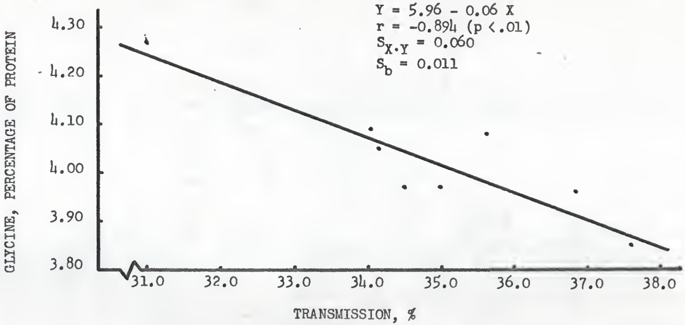

Fig. 17. The correlation between percent transmission and the percent glycine in protein of Hard Red Winter wheat.

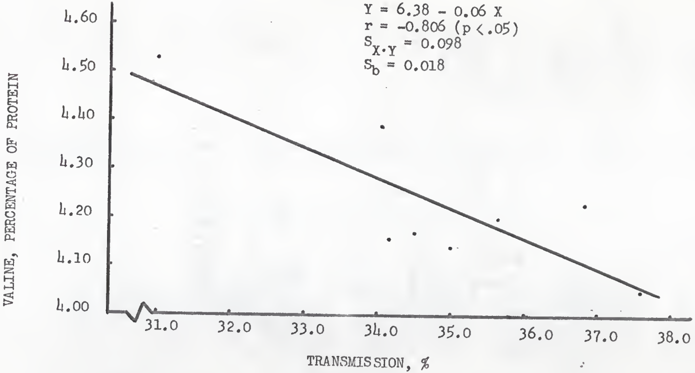

Fig. 18. The correlation between percent transmission and percent valine in protein of Hard Red Winter wheat.

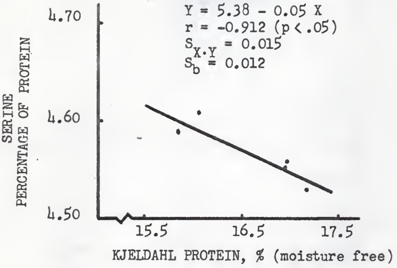

Fig. 19. The correlation between Kjeldahl protein content and percent serine in protein of Northern Spring wheat.

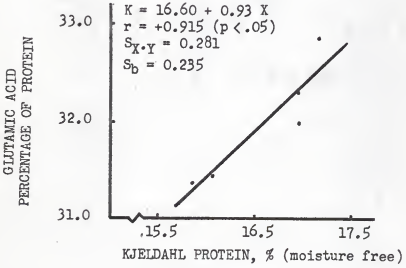

Fig. 20. The correlation between Kjeldahl protein content and the percent glutamic acid in protein of Northern Spring wheat.

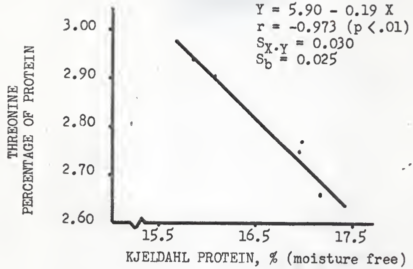

Fig. 21. The correlation between Kjeldahl protein content and the percent threonine in protein of Northern Spring wheat.

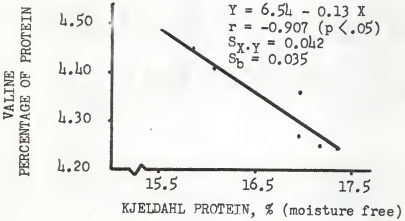

Fig. 22. The correlation between Kjeldahl protein content and percent valine in protein of Northern Spring wheat.

AMINO ACID COMPOSITION OF WHEAT AS RELATED TO DYE ABSORPTION

by

FLOYD KENT SHOUP

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Sixteen pure varieties of wheat were obtained from various areas in the United States. The amino acid composition of the wheats and their flours was determined using a Beckman Model 120B Amino Acid Analyzer. The amino acids analyzed for were lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, half cystine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine. The amino acids are reported as grams of amino acid in 100 grams protein calculated from the analyzer.

Protein determinations were made on the 16 composites by the Kjeldahl procedure. The samples were also reacted with acid orange 12 dye to determine the amount of dye bound by the protein. Light transmittance was determined on the unbound portion of the dye.

Little or no differences in amino acid concentration were observed among varieties of wheat. Those small differences noted possibly could have arisen due to the variation in the protein content between varieties.

Studies of the amino acid composition indicated differences between wheat and its flour. The differences found between the Hard Red Winter wheat and its flour parallel very closely those found in the Northern Spring wheats. The milling of the wheat into flour lowered the amino acid concentration in the protein for the following amino acids in both the Hard Red Winter and Northern Spring wheats: lysine, histidine, arginine, aspartic acid, threonine, glycine, alanine, valine, and tyrosine. Those which were more concentrated in the flour protein for both classes of wheat were glutamic acid, proline, and phenylalanine. Half cystine and isoleucine concentrations were somewhat increased in the Hard Red Winter wheat flour while methionine was substantially increased in the flour of the Northern Spring wheat.

A positive correlation was observed between Kjeldahl protein[1] content and percent transmittance of the unbound dye as determined by the Udy instrument when using data from all 16 varieties of wheat. When the Hard Red Winter wheat data were considered alone, there was a significant positive correlation between Kjeldahl protein content and percent transmittance. No significant correlation was found, however, when using the data from the Northern Spring composites.

Kjeldahl protein[2] content was found to be negatively correlated with the sum of the basic amino acids[3] lysine, histidine, and arginine by use of data from each of the 16 varieites of wheat. However, when the 16 composites were divided into the two classes, Hard Red Winter wheat and Northern Spring wheat, no significant correlation was observed between Kjeldahl protein and the sum of the basic amino acids.

Each of the 17 amino acids was considered as a function of Kjeldahl protein. The unit of amino acid used in the computation of all subsequent

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[1] The Kjeldahl protein was not corrected for moisture.

[2] The values for Kjeldahl protein used in this correlation and in following correlations will be corrected to zero moisture content.

[3]

$$\text{Basic amino acids as percentage of protein} = \frac{\text{Summation of the grams of lysine, histidine and arginine}}{\text{Total grams of amino acids and ammonia accounted for by the amino acid analyzer}} \times 100$$

correlations will be expressed as percentage of the protein.[4]

Correlations were calculated using data for the Hard Red Winter wheat composites. The amino acids which were found to have significant negative correlations were threonine, serine, glycine, half cystine, methionine, and valine. Glutamic acid had a significant positive correlation. The same statistical analysis was made using the data from the Northern Spring wheats. Threonine, serine, and valine were found to be negatively correlated. Glutamic acid was again positively correlated.

Correlations calculated to determine the relationship of percent transmittance of unbound dye and the sum of the basic amino acids were nonsignificant for both Hard Red Winter wheat and Northern Spring wheat.

A study was made to determine possible correlations between dye binding and the individual amino acids. The Hard Red Winter wheat composites were found to have significant negative correlations with: threonine, serine, glycine, half cystine, valine and methionine. Glutamic acid had a significant positive correlation.

When correlations were calculated using the Northern Spring wheat data, none were found to be significant.

The data reported in this paper are preliminary in character. However, they do indicate a need for further research into the relationships between dye binding and amino acid composition.

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[4]

$$\text{Amino acid, percentage of protein} = \frac{\text{Grams of the amino acid}}{\text{Total grams of amino acids and ammonia accounted for by the amino acid analyzer}} \times 100$$